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# Significance of Mitochondrial DNA Haplogroup on Epidermal Growth Factor Receptor Mutation in Japanese Patients With Lung Adenocarcinoma

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**Abstract.** *Background/Aim: The frequency of somatic mutations of epidermal growth factor receptor (EGFR) in primary lung adenocarcinoma varies among populations and countries. The aim of the present study was to clarify whether the frequency of EGFR mutations in patients with lung adenocarcinoma depends on their mitochondrial DNA haplogroup, which reflects their maternal lineage. Patients and Methods: Using normal lung tissue specimens, the mitochondrial DNA haplogroup was determined by multiplex polymerase chain reaction in 135 Japanese patients who underwent surgery for primary lung adenocarcinoma. Results: The 135 patients were divided into two groups according to the two primitive haplotypes (N group, n=32; M group, n=103). The frequency of EGFR mutations in the N group was significantly higher than that in the M group (69% vs. 48%, p=0.044). The difference was prominent when the analysis was restricted to non-smokers (95% vs. 57%, p<0.01). Conclusion: The frequency of EGFR mutations in lung adenocarcinoma patients depends on their mitochondrial lineage.*

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*Key Words:* Mitochondrial DNA haplogroup, *EGFR* mutation, lung cancer.

Somatic mutations of the epidermal growth factor receptor gene, *EGFR*, are known to be major driver mutations that contribute to the development and progression of non-small cell lung cancer, particularly adenocarcinoma. In patients with unresectable adenocarcinoma harboring *EGFR* mutations, EGFR tyrosine kinase inhibitor is the first-line treatment, with a response rate of >50% (1). The proportion of *EGFR* mutation-positive lung adenocarcinoma among total lung adenocarcinoma varies by population and country, and the underlying mechanisms remain unknown: *EGFR* mutations are detected in ≥50% of individuals in East Asian populations, but <20% in North American and European populations (2), indicating that the genetic background is involved in the etiology. The identification of patients who are highly susceptible to *EGFR* mutation-positive lung cancer would be beneficial in the search for the responsible genetic components by the comparison of genomes between the populations using next-generation sequencing. We hypothesized that the susceptibility to *EGFR* mutation varies in individual patients according to their mitochondrial deoxyribonucleic acid (mtDNA) haplogroup, which specifically reflects maternal lineage (mtDNA is exclusively inherited from the mother). As shown in the relevant literature, human ancestors who moved out from Africa are divided into two major lineages: haplogroups N and M (3). Each haplogroup is further divided into multiple lineages as humans spread throughout the world (3). In the present study, we examined whether the frequency of *EGFR* mutations in patients with lung adenocarcinoma depends on their mtDNA haplogroup.

Table I. *Characteristics of the patients according to the mitochondrial DNA haplogroup (n=135).*

| Variables | | Total (n=135) | Haplogroup | | *p*-Value |
|---|---|---|---|---|---|
| | | | N (n=32) | M (n=103) | |
| Age | Years | 67.6±9.4 | 69.7±7.2 | 67.0±10.0 | 0.159 |
| Gender | Male/Female | 65/70 | 16/16 | 49/54 | 0.842 |
| Smoking | Ever/Never | 60/75 | 13/19 | 47/56 | 0.686 |
| Pack years | Packs | 18.4±26.9 | 15.2±23.7 | 19.5±27.8 | 0.815* |
| Laterality | Right/Left | 71/64 | 18/14 | 53/50 | 0.688 |
| Location | Upper/Lower | 87/48 | 20/12 | 67/36 | 0.834 |
| Tumor size | mm | 25.2±12.5 | 24.4±13.1 | 25.5±12.4 | 0.678 |
| Lepidic growth | Yes/No | 64/71 | 18/14 | 46/57 | 0.312 |

*Non-parametric test.

## Patients and Methods

*Patients*. This study was approved by our institutional review board and written informed consent was obtained from all patients included in this study (No. 26-159). We identified 135 Japanese patients with pathologically proven primary lung adenocarcinoma who underwent surgery at our institution between January 2015 and December 2017. The demographic variables obtained before surgery included age, sex, pack-years smoked, affected side (right *vs*. left), tumor location (upper *vs*. lower lobe), maximum tumor diameter, and presence or absence of lepidic growth pattern. The characteristics of the 135 patients are shown in Table I.

*Tissue sampling*. During surgery, the normal lung tissue was obtained immediately after lung resection and was stored at -80°C until the extraction of DNA. The tumor tissue was preserved in paraffin blocks after formalin fixation.

*Assessment of EGFR mutations*. We analyzed the *EGFR* mutation status of formalin-fixed paraffin-embedded specimens. The mutation status of *EGFR* exons 18, 19, 20 and 21 was examined using the peptide nucleic acid-locked nucleic acid polymerase chain reaction (PCR) clamp method (4). This method can detect known mutations (G719A, G719C, and G719A in exon 18; all deletion genotypes in exon 19; T790M in exon 20; and L858R and L861Q in exon 21) using PCR primers, each of which is designed for a specific mutated sequence (4).

*Haplotype determination*. Genomic DNA was extracted from the resected lung tissue with a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). Haplotypes of mtDNA were determined based on the DNA sequence of mtDNA hypervariable region 1 (HV1), as determined by singleplex PCR or multiplex PCR. The following primers were used in singleplex PCR: 548-F, 5'-TGTAA AACGACGGCCAGTCTCCACCATTAGCACCCAAAGCT-3' and 548-R, 5'-CAGGAAACAGCTATGACCTGGCCCTGAAGTAG GAACCAGA-3', they contain the M13 forward and reverse sequences, respectively. The primers were used to amplify the 548 bp fragment (15,974 to 16,521 bp), which covers part of the non-coding region and HV1. The amplified region was sequenced using a BigDye® Terminator v1.1 cycle sequencing kit (Thermo Fisher Scientific, Cleveland, OH, USA) with M13 forward and reverse primers and an Applied Biosystems 3500Dx Genetic Analyzer (Thermo Fisher Scientific, Inc.). The sequences were analyzed using a Sequence Scanner (Applied Biosystems Inc, Framingham, Mass), with the revised Cambridge Reference Sequence (rCRS; GenBank accession number NC_012920.01) as a reference. According to the patients' mtDNA sequences, we determined the B4, M7a, A, F, N9a, M7b, B5, M9, M10, Z, M7c, C and Y haplotypes by referring to the available mtDNA database (5, 6). To determine other haplotypes, we performed 2 groups of multiplex PCRs using KOD-Multi & Epi (TOYOBO Co., Ltd., Osaka, Japan). Group 1 contained the D5 and M8a primer sets and group 2 contained the G, D4 and N9b primers. Each polymorphism was determined by the PCR product size (Table II). The M8a, G, D4 and N9b primers have been reported previously (7), the D5 primer set was designed to distinguish the 1107C polymorphism.

*Statistical analysis*. The values are expressed as the mean±standard deviation (SD). An unpaired *t*-test or nonparametric test was used to test continuous variables. Fisher's exact test was used to compare categorical variables. *p*-Values of <0.05 were considered to indicate statistical significance. Univariate and multivariate logistic regression analyses were used to determine the relationship between various factors and the presence of EGFR mutations. A multivariate regression analysis that included all variables with a *p*-value of <0.1 was performed to identify independent predictors of *EGFR* mutation. All statistical analyses were performed using the SPSS software program (SPSS statistics version 22, IBM, Armonk, NY, USA).

## Results

*EGFR* mutations were detected in 71 of the 135 (53%) patients. *EGFR* L858R (exon 21) was the most frequent type of mutation (41 of 71 patients) followed by *EGFR* exon 19 deletion (25 of 71 patients) and other mutations (5 of 71 patients).

Based on the mitochondrial DNA sequencing, we identified 19 mtDNA haplogroups in the 135 patients (Table III). These 19 mtDNA haplogroups could be categorized into 9 mtDNA composite haplogroups according to their first alphabetic descriptors for mtDNA. These 9 composite

Table II. *Primers used in the multiplex polymerase chain reaction to determine mitochondrial DNA haplogroup.*

| PCR | Primer sets | Primer | Sequence | PCR product size (bp) | Haplogroup |
|---|---|---|---|---|---|
| Group 1 | D5 | F(1107T) | CACTATGCTTAGCCCTAAACCT | 105 | |
| | | F(1107C) | GTTTAACTATGCTTAGCCCTAAACCC | 109 | D5 |
| | | R(1107R) | GATATGAAGCACCGCCAGGT | | |
| | M9a | F(8684F) | CGACTAATCACCACCCAAC | | |
| | | R(8684T) | ACAATGGrTATCATTTGTTATTGAGA | 62 | M9a |
| | | R(8684C) | TGGTATCATTTGATTTGAGG | 58 | |
| Group 2 | G | F(4833A) | TCAAACAGAGGTTACCCTAGGCA | 114 | |
| | | F(4833G) | CAGAGGTTACCCATGGCG | 109 | G |
| | | R(4833R) | CTTACGTTTAGTGAGGGAGAG | | |
| | D4 | F(3010A) | GCTACATGGATCAGGACAACCCA | 95 | D4 |
| | | F(3010G) | ATTGGATCAGGACTTCCCG | 91 | |
| | | R(3010R | TTCCGGTCTGAACTCAGATC | | |
| | N9b | F(13183F) | ATCCAAACTCTAACACTATGCTTAGG | 53 | |
| | | R(13183A) | TATGCTGCGAACAGAGAGGTGAT | 50 | N9b |
| | | R(13183G) | AATGCGAACAGAGTGGAGAC | | |

PCR: Polymerase chain reaction. F and R in primer indicate forward and reverse primer.

Table III. *Distribution of mitochondrial DNA haplogroups in 135 patients.*

| Macrohaplogroup | N | | | | | | | | M | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. of patients | 32 | | | | | | | | 103 | | | | | | | | | | |
| Composite Haplogroup | A | B | | | F | H | | N | C | D | | | G | M | | | | | |
| No. of patients | 9 | 13 | | | 4 | 2 | | 4 | 3 | 67 | | | 11 | 22 | | | | | |
| Haplogroup | A | B2 | B4 | B5 | F | H2 | H5 | N9a | C | D4 | D5 | D6 | G | M7a | M7b | M8a | M9 | M10 | M11 |
| No. of patients | 9 | 1 | 9 | 3 | 4 | 1 | 1 | 4 | 3 | 64 | 2 | 1 | 11 | 9 | 2 | 3 | 3 | 2 | 3 |

mtDNA haplogroups could be eventually summarized into 2 mtDNA macrohaplogroups (haplogroups N and M) (Table III, Figure 1). Table I shows the comparison of the characteristics of patients regarding the mtDNA haplogroups N and M. No significant difference was observed between the groups. Table IV shows the rate of *EGFR* mutation positivity in each of the 9 mtDNA haplogroups. The positive rates varied greatly among the different mtDNA haplogroups, ranging from 36% to 89%. Table IV also shows the rates of *EGFR* exon 19 mutation positivity, *EGFR* exon 21 mutations, and other minor mutations. When patients were grouped into two haplogroups (N or M), the rates of positivity for all *EGFR* mutations were significantly higher in patients with mtDNA haplogroup N in comparison to patients with mtDNA haplogroup M (69% *vs*. 48%, $p$=0.044). With respect to the *EGFR* mutation subtypes, the rate of *EGFR* exon 19 mutation positivity in patients with mtDNA haplogroup N was comparable to that in patients with mtDNA haplogroup M (16% *vs*. 19%, $p$=0.80). In contrast, the rate of *EGFR* exon 21 mutation positivity in patients with mtDNA haplogroup N was significantly higher than that in patients with mtDNA haplogroup M group (50% *vs*. 24%, $p$=0.008). To exclude the effect of tobacco exposure, we analyzed selected patients who did not have smoking history (n=75). As a result, the extent of difference in the rate of *EGFR* mutation positivity between the mtDNA haplogroup N and M became prominent: the positive rate for any *EGFR* mutation was significantly higher in patients with mtDNA haplogroup N than in patients with mtDNA haplogroup M group (95% *vs*. 57%, $p$=0.002) (Table V). With respect to *EGFR* mutation subtype, the rate of *EGFR* exon 19 mutation positivity in patients with mtDNA haplogroup N was comparable to than in patients with mtDNA haplogroup M group (21% *vs*. 21%, $p$=1.00) (Table V). In contrast, the rate of *EGFR* exon 21 mutation positivity was significantly higher in patients with mtDNA haplogroup N than in patients with mtDNA haplogroup M group (68% *vs*. 32%, $p$=0.007) (Table V). These results are also shown in a Rader chart (Figure 2).

In order to clarify the association between mtDNA haplogroup (N or M) and the frequency of *EGFR* mutations,

Table IV. *The incidence of epidermal growth factor receptor mutation types according to the mitochondrial DNA haplotypes (haplogroups) in overall patients (n=135).*

| Mutation type | Total | N haplogroup | | | | | M haplogroup | | | | *p*-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | F | H | N | C | D | G | M | |
| Any | 71/135 | | | 22/32 | | | | | 49/103 | | 0.044 |
| (%) | (53%) | | | (69%) | | | | | (48%) | | |
| | | 8/9 | 8/13 | 2/4 | 1/2 | 3/4 | 2/3 | 34/67 | 4/11 | 9/22 | |
| | | (89%) | (62%) | (50%) | (50%) | (74%) | (67%) | (51%) | (36%) | (41%) | |
| Exon 19 | 25/135 | | | 5/32 | | | | | 20/103 | | 0.796 |
| (%) | (19%) | | | (16%) | | | | | (19%) | | |
| | | 1/9 | 1/13 | 0/4 | 1/2 | 2/4 | 2/3 | 12/67 | 3/11 | 3/22 | |
| | | (11%) | (8%) | (0%) | (50%) | (50%) | (67%) | (18%) | (27%) | (14%) | |
| Exon 21 | 41/135 | | | 16/32 | | | | | 25/103 | | 0.008 |
| (%) | (30%) | | | (50%) | | | | | (24%) | | |
| | | 7/9 | 6/13 | 2/4 | 0/2 | 1/4 | 0/3 | 19/67 | 1/11 | 5/22 | |
| | | (78%) | (46%) | (50%) | (0%) | (25%) | (0%) | (28%) | (9%) | (23%) | |
| Others | 5/135 | | | 1/32 | | | | | 4/103 | | 1.000 |
| (%) | (4%) | | | (3%) | | | | | (4%) | | |
| | | 0/9 | 1/13 | 0/4 | 0/2 | 0/4 | 0/3 | 3/67 | 0/11 | 1/22 | |
| | | (0%) | (8%) | (0%) | (0%) | (0%) | (0%) | (4%) | (0%) | (5%) | |

Table V. *The incidence of epidermal growth factor receptor mutation types according to the mitochondrial DNA haplotypes (haplogroups) in non-smokers (n=75).*

| Mutation type | Total | N haplogroup | | | | | M haplogroup | | | | *p*-Value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | F | H | N | C | D | G | M | |
| Any | 71/135 | | | 18/19 | | | | | 32/56 | | 0.002 |
| (%) | (53%) | | | (95%) | | | | | (57%) | | |
| | | 6/6 | 7/8 | 2/2 | 1/1 | 2/2 | 0/0 | 26/42 | 2/3 | 4/11 | |
| | | (100%) | (88%) | (100%) | (100%) | (100%) | | (62%) | (67%) | (36%) | |
| Exon 19 | 25/135 | | | 4/19 | | | | | 12/56 | | 1.000 |
| (%) | (19%) | | | (21%) | | | | | (21%) | | |
| | | 1/6 | 1/8 | 0/2 | 1/1 | 1/2 | 0/0 | 9/42 | 1/3 | 2/11 | |
| | | (17%) | (13%) | (0%) | (100%) | (50%) | | (21%) | (33%) | (18%) | |
| Exon 21 | 41/135 | | | 13/19 | | | | | 18/56 | | 0.007 |
| (%) | (30%) | | | (68%) | | | | | (32%) | | |
| | | 5/6 | 5/8 | 2/2 | 0/1 | 1/2 | 0/0 | 15/42 | 1/3 | 2/11 | |
| | | (83%) | (63%) | (100%) | (0%) | (50%) | | (36%) | (33%) | (18%) | |
| Others | 5/135 | | | 1/19 | | | | | 2/56 | | 1.000 |
| (%) | (4%) | | | (5%) | | | | | (4%) | | |
| | | 0/6 | 1/8 | 0/2 | 0/1 | 0/2 | 0/0 | 2/42 | 0/3 | 0/11 | |
| | | (0%) | (3%) | (0%) | (0%) | (0%) | | (5%) | (0%) | (0%) | |

we performed univariate and multivariate logistic regression analyses. According to the univariate analysis, female sex, light or never smoking, upper lobe cancer, lepidic growth tumor, and mtDNA haplogroup N were positively associated with the presence of *EGFR* mutation (Table VI). According to a multivariate analysis that included these variables, light or no smoking, upper lobe cancer, and mtDNA haplogroup N showed a significant association with *EGFR* mutation (Table VI).

## Discussion

The frequencies of somatic mutations of *EGFR* in primary lung adenocarcinoma vary depending on country, region, and

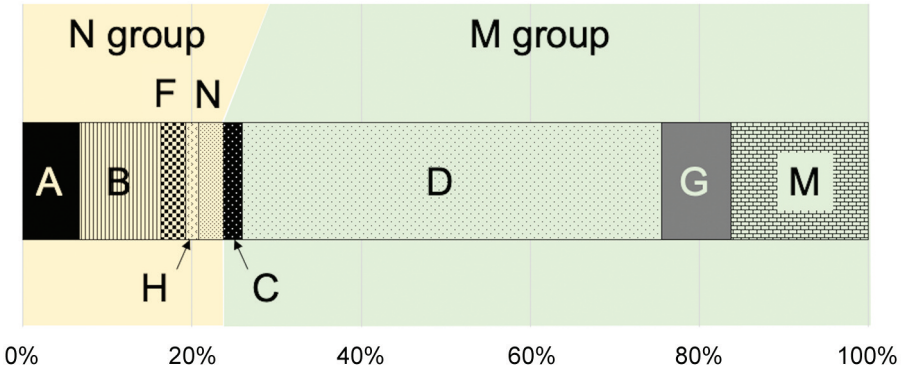


Figure 1. *Frequency distribution of mitochondrial DNA haplogroups in 135 Japanese patients with lung adenocarcinoma.*

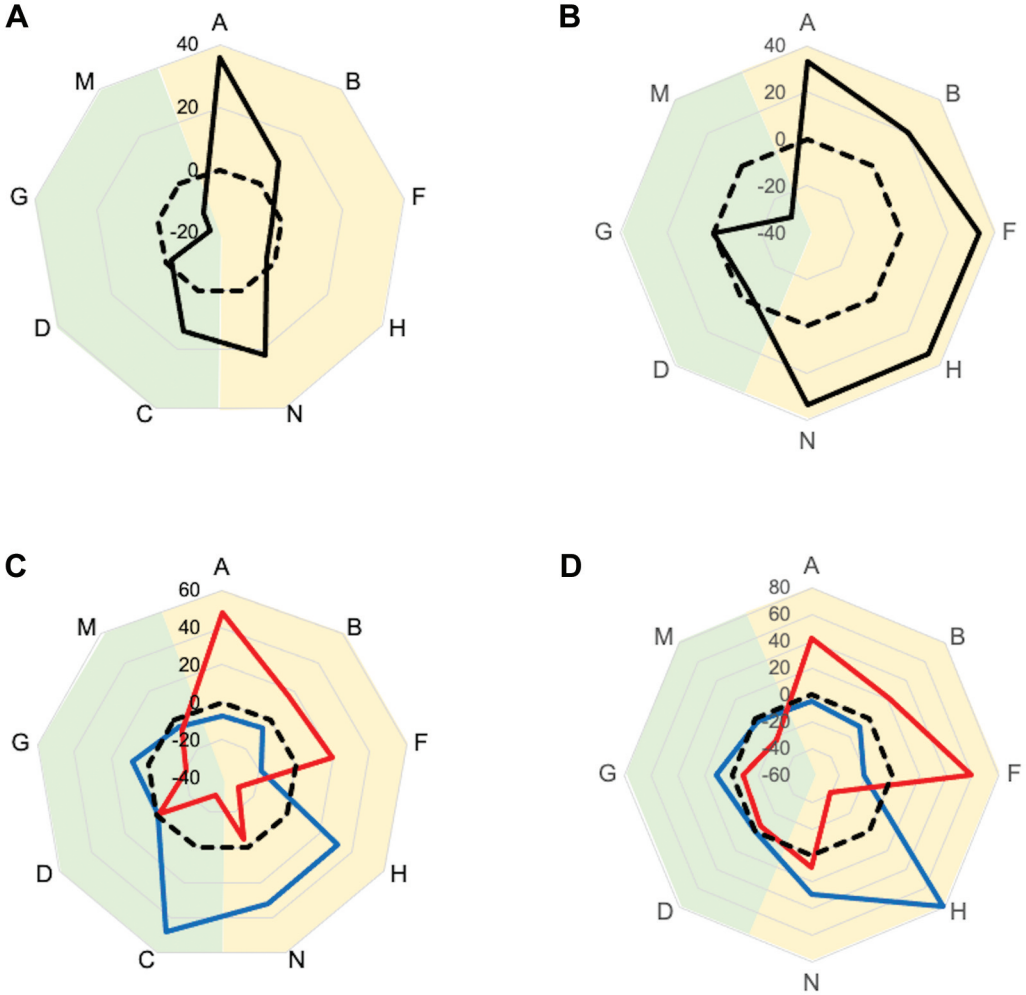


Figure 2. *Upper panels show a Rader chart of the rate of EGFR mutations in each mitochondrial DNA haplogroup of 135 lung adenocarcinoma patients (A) and in 75 lung adenocarcinoma patients without a smoking history (B). Solid lines indicate the percentage of EGFR mutations above the mean (dotted lines). Lower panels show the Rader chart of the rate of EGFR exon 19 mutation (red line) and exon 21 mutation (blue line) in each mitochondrial DNA haplogroup of 135 lung adenocarcinoma patients (C) and in 75 lung adenocarcinoma patients without a smoking history (D). Solid lines (red and blue) indicate the percentage of EGFR mutation subtypes above the mean (dotted lines).*

Table VI. *Univariate and multivariate analyses for indicators of epidermal growth factor receptor mutation.*

| Variables | Reference | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| | | OR | 95%CI | *p*-Value | OR | 95%CI | *p*-Value |
| Age | 1 year | 1.007 | 0.971-1.044 | 0.710 | | | |
| Gender | Female | 0.420 | 0.210-0.838 | 0.014 | | | |
| Pack years | 1 pack | 0.967 | 0.950-0.984 | <0.001 | 0.968 | 0.951-0.985 | <0.001 |
| Laterality | Left | 1.082 | 0.550-2.128 | 0.820 | | | |
| Location | Lower lobe | 2.598 | 1.257-5.369 | 0.010 | 2.438 | 1.107-5.369 | 0.027 |
| Tumor size | 1 mm | 0.993 | 0.966-1.020 | 0.595 | | | |
| Lepidic growth | Yes | 0.279 | 0.137-0.569 | <0.001 | | | |
| Mitochondrial DNA haplogroup | M | 2.424 | 1.045-5.625 | 0.039 | 2.615 | 1.021-6.695 | 0.045 |

population. In the present study, we hypothesized that the susceptibility to *EGFR* mutation varies in individuals according to their mtDNA haplogroup, which specifically reflects their geographic ancestry. We demonstrated that the rate of *EGFR* mutation positivity in patients with mtDNA haplogroup N was significantly higher in comparison to those with mtDNA haplogroup M. When an analysis was performed separately for each *EGFR* mutation subtype (exons 21 and 19), the significant difference was still observed in the frequency of *EGFR* exon 21 mutations, but not in the frequency of *EGFR* exon 19 mutations. With respect to the factors predicting *EGFR* mutations, a previous report suggested a significant relationship between *EGFR* mutations and various cancer phenotypes, patient demographic factors, and environmental factors (8). In our patients, smoking history, sex, site of primary lesion, presence or absence of lepidic growth pattern, and mtDNA haplogroup were significantly associated with *EGFR* mutation in the univariate analysis. In the multivariate analysis, mtDNA haplogroup, smoking history, and site of lesion were found to be significant independent predictors of *EGFR* mutation. To the best of our knowledge, this is the first report on the relationship between mtDNA haplogroups and *EGFR* mutation in primary lung cancer.

Previous reports suggested a significant association between mtDNA haplogroups and susceptibility to some types of carcinomas or the likelihood of cancer relapse. For instance, mtDNA haplogroup N has been associated with an increased risk of breast cancer and esophageal squamous cell cancer (9). In addition, mtDNA composite haplogroups C, Z, and D have been associated with favorable relapse-free survival in patients with oral squamous cell carcinoma (10). Irrespective of these novel findings, the underlying mechanisms remain to be elucidated. Multiple processes are involved in the development and progression of cancer, and thereby participate in multiple pathogenic molecular pathways, making it difficult to focus on the responsible alterations in mtDNA. In addition, it remains unclear whether or not these results, regarding mtDNA haplogroups and cancer, can be reproduced in a validation study with different populations from different countries or races. In the present study, we focused exclusively on the relationship between EGFR mutation and mtDNA haplogroup in primary lung adenocarcinoma. We found close relationships between them, particularly in non-smokers. However, the reproducibility of the current study should be explored in different populations from different countries, such as Peru or Vietnam, in which the frequency of *EGFR* mutations is prominently high (2).

With regard to the *EGFR* mutation subtypes, the frequency of *EGFR* exon 21 mutation positivity was significantly correlated with the mtDNA haplogroup, while the frequency of *EGFR* exon 19 mutation was not. According to a previous report, the frequency of *EGFR* mutation (any mutation) was highest in the Asian population, followed by Latinos, non-Latino whites, and non-Latino blacks (11). However, the ratio of patients with *EGFR* exon 21 mutation to those with *EGFR* exon 19 mutation did not differ among the populations. Although the reason why exon 21 mutation, but not exon 19 mutation, was associated with mtDNA haplogroup remains unknown. It is possible that certain genotypes confer susceptibility to *EGFR* exon 21 mutation, but not *EGFR* exon 19 mutation.

A previous study revealed genetic factors underlying the risk of *EGFR* mutation-positive lung adenocarcinoma by conducting a genome-wide association study using germline DNAs from Japanese patients (12). Several single-nucleotide polymorphisms (SNPs) in the HLA-class II and FOXP4 regions were identified as being significantly associated with *EGFR* mutation-positive lung adenocarcinoma. For instance, the odds ratio of HLA-class II polymorphisms in the development of *EGFR* mutant lung adenocarcinoma was 1.36. Based on these results, it may be valuable to determine the correlation between mtDNA haplogroups and germline DNA polymorphisms of HLA-class II and FOXP4. In a

recent study a genome-wide scan was performed to assess the genotype distribution dependence between mtDNA and nuclear DNA variants using whole genome sequencing data (13). In this study, multiple comparisons revealed no significant mtDNA–nuclear DNA genotype association, which partly contradicts our hypothesis regarding the dependence between the mtDNA haplogroup and nuclear DNA polymorphisms. However, that comparison of highly selected populations based on mtDNA haplogroup could help identify the responsible genotype in germline DNAs that confers high susceptibility to EGFR mutant lung adenocarcinoma in the Japanese population.

Other possible explanations of the significance of the mtDNA haplogroup and *EGFR* mutation status may be that mitochondrial genotype alterations in haplogroup N can directly or indirectly contribute to the promotion of *EGFR* mutation, particularly in exon 21. The mitochondrion is the site of ATP synthesis for the cell, and thus, related to the metabolic activity of cells. The mitochondria also have a role in maintaining the intracellular environment, apoptosis, and calcium storage (14). Thus, mutations in the coding regions of mitochondrial DNA can cause various pathological conditions, including inherited diseases known as mitochondrial diseases. Interestingly, whether or not a SNP in mtDNA leads to a pathological phenotype depends on the patient's mtDNA haplogroup (15). Thus, we must perform a further genome-wide scan of mtDNA to assess dependence among mtDNA genotype, mtDNA haplogroup, and *EGFR* mutation status.

According to a comprehensive genomic analysis that was performed in a Japanese nationwide project, the composition of mtDNA haplogroups in the Japanese population varies in different regions (12). Overall, the most frequent mtDNA haplogroup was haplogroup D, followed by M, B, G, and A, which is in accordance with our results. Nevertheless, we clearly demonstrated that the rate of *EGFR* mutation positivity was remarkably high in non-smokers belonging in mtDNA haplogroup N (18 of 19 patients; 95%) in comparison to smokers (32 of 56 patients; 57%, $p$=0.002).

In conclusion, the incidence of *EGFR* mutations in patients with lung adenocarcinoma depends on the maternal lineage. Further study to identify the genetic alterations responsible for *EGFR* mutation in patients with haplogroup N is warranted.

## Conflicts of Interest

The Authors declare no conflicts of interest in association with the present study.

## Authors' Contributions

All Authors take responsibility for the integrity of the data and the accuracy of the data analysis. OT, FT, and SM contributed to the study design, data collection, and biological assessment. All Authors contributed substantially to the data analysis and interpretation. UK, OT, and SM contributed to the writing of the manuscript. All Authors read and approved the manuscript.

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